SHELL ULTRASTRUCTURE IN ALLOGROMIID FORAMINIFERA (PROTOZOA)

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INTRODUCTION

The form and structure of calcified or agglutinated foraminiferal shells are important in the classification of Foraminiferida and, as a consequence, have been the subject of extensive studies. In contrast, the structure of tectinous or purely organic foraminiferal shells has received relatively less attention. Since the subject was reviewed by Hedley (1964) a number of morphological studies of tectinous forms, using transmission electron microscopy, have been published which include brief descriptions of shell-structure: for example, *Shepheardella taeniformis* (Hedley *et al.*, 1967), *Allogromia laticollaris* (Lengsfeld, 1969; Schwab, 1970) and *Myxotheca arenilega* (Schwab, 1969; Angell, 1971).

The present account describes the shell-structure of six additional tectinous foraminiferans and attempts to relate some aspects of the fine structure to the functioning of the shell in the living animal.

PREVIOUS WORK

The ability of some tectinous foraminifera to alter their shape appears to have been initially reported by Cushman (1920), after examining *Iridia diaphana.* Morphological variants of *Allogromia laticollaris* are described by Arnold (1954a), who reported that young animals appear to be more flexible than older individuals. Recently freed schizonts, for example, are characterised by erratic distension and contraction at localised points at the periphery of the shell. Similar movements have been observed in an unnamed *Allogromia* sp., which varied in shape from ovoid to elongate, bioral, '*Shepheardella*-like' forms (Lee & Pierce, 1963). These authors note that such irregular forms are very plastic and continually change shape. *Shepheardella taeniformis* and *Boderia turneri* are capable of great and rapid change in form (Hedley, 1964 and Hedley *et al.*, 1968).

Wohlfarth-Bottermann (1961) in an account of the fine-structure of *Allogromia laticollaris* comments on the occurrence of fine pores in the shell with cytoplasmic threads running through them. He does not, however, comment on the nature or structure of the shell wall material itself. Lengsfeld (1969) describes the shell of *A. laticollaris* as being composed of filament-like elements embedded in a glue-like substance; the filaments being arranged parallel to the surface of the cell with the outermost layer appearing to be coarsely granular. Schwab (1969) describes the shell of both young and adult specimens of *Myxotheca arenilega* a being composed of fibrous-like material. In adult specimens the wall is divided into two layers of unequal thickness, the outer layer being thin and osmophilic, whilst the inner layer

is thick and composed of fibrous material lying parallel to the cell surface. He further suggests that this material is formed in the Golgi apparatus. A report by Angell (1971) describes the test of *Myxotheca* sp. as being composed of fibrous material, of undetermined chemical composition, but having a 'herring-bone' pattern. He also suggests that these fibres originate from vacuoles at the inner edge of the test wall. The shell of *Shepheardella taeniformis* is described by Hedley *et al.* (1967) as having a fibrous component which is not recognised as being orientated in any way. In a detailed study of the fine structure of *Gromia oviformis*, which occupies a rather enigmatic position in the classification of the Protozoa, the organic shell consists of three distinct regions (Hedley & Wakefield, 1969). These are an outer region composed of fine fibres with an electron-dense outer layer. a middle region of complex honeycombed membranes and an inner region of fibrillar material. The fine fibrillar elements of both the inner and outer regions appear to be morphologically similar.

Numerous workers (Moss, 1963; Hedley, 1962, 1963; Pierce *et al.*, 1968; Angell, 1967 and Hedley & Wakefield, 1969) recognise the organic wall of the tectinous foraminifera as being composed of protein and acid mucopolysaccharides. Attempts have been made to define the composition of some of these organic shells using cytochemical methods (Angell, 1967, and Pierce *et al.*, 1968) and amino-acid analysis (Hedley & Wakefield, 1969). Nevertheless, the precise composition remains obscure because of the technical difficulties in isolating this material from the cytoplasm of the animal, and because of the limited value of the cytochemical and other methods employed so far.

MATERIALS AND METHODS

The animals used and discussed in this paper are as follows:

Allogromia laticollaris Arnold, 1948

—maintained in this laboratory for several years from cultures established from collections from Panama City, Florida, U.S.A. by Professor Z. M. Arnold.

Allogromia sp. A and B

—isolated from tufts of the inter-tidal calcareous alga *Corallina officinalis* at P ymouth, England

Allogromia sp. C

—isolated from tufts of *Corallina officinalis* from Wellington, New Zealand.

Iridia diaphana Heron-Allen & Earland, 1914

—isolated from sandy sediment, 5 fm, from Plymouth, England.

Boderia turneri (Wright, 1867)

—isolated from tufts of *Corallina officinalis* from Plymouth, England.

The cultures are kept at room temperature, 18–20°C, in small plastic containers and maintained on Foyn's Erdschreiber medium (Arnold, 1954b). The animals are sub-cultured regularly every five or six weeks and are fed weekly on freshly killed *Tetraselmis chui*. Under these conditions the animals appear to feed and reproduce readily, with the exception of *Allogromia* sp. A, *Boderia turneri* and *Iridia diaphana* which remain alive and active for about four months, but do not appear to grow or

reproduce under these laboratory conditions. We are unable to distinguish specifically between the three forms A, B and C of *Allogromia* but the characters which appear to be significant are detailed in Table 1.

Electron Microscopy

The standard fixation and embedding used for these species is as follows. Specimens are fixed in 4 per cent glutaraldehyde in 0·1 M cacodylic acid buffer with 0·25 M sucrose for 3 h at pH 7·0. After several washes in buffer, they are post-fixed in Caulfield's osmium tetroxide followed by rapid ethanol dehydration and finally embedded in Epon 812. One naked specimen of *Iridia diaphana* which had left its 'tent' and was observed to become an elongate form (Text-fig. 1), was fixed *in situ* in the plastic culture vessel with 4 per cent glutaraldehyde in equal parts of 0·1 M cacodylic acid buffer with 0·25 M sucrose and sea-water, pH 7·4, for forty-five minutes, washed with several changes of equal parts of buffer and sea-water, post-fixed with 1 per cent osmium tetroxide in 0·1 M cacodylic acid buffer, followed by rapid ethanol dehydration and embedded in Epon. Sections were cut with a diamond knife (Du Pont) on a Porter-Blum Mt 2 ultramicrotome, stained with a saturated alcoholic solution of uranyl acetate followed by Reynold's lead citrate. All the micrographs were obtained from an AEI EM 6B microscope operating at 60 kV and recorded on either Ilford's N50 or 'special lantern contrasty' plates.

RESULTS

Allogromia laticollaris

The shell appears to be divided into two distinct regions: an outer, thin, electron-dense region 0·4 to 1·0 μm thick and an inner fibrillar region 1·8 to 3·0 μm thick (Pl. 1, fig. A). In those animals examined the shell varies in thickness from 2 to 4 μm, but it can vary between 4 and 38 μm in thickness, according to Pierce *et al.* (1968). The outer region is composed of concentrations of electron-dense material with a fine fibrillar network interspersed. The inner region is composed of a mass of fine fibrillar material which appears to be organized into layers. In cross-sections of the wall the fibrils appear to be aligned parallel to the cell surface, and occasionally they are arranged to produce a 'herring-bone' pattern (Pl. 1, fig. A). In tangential section the fibrils appear to be arranged at random. The fibrils are approximately 4 nm thick and the electron-dense points are approximately 14 nm in diameter. The diameter of these points is similar to that found in the electron-dense region.

Allogromia sp. A.

The shell is divided into three distinct regions, an outer, intense, electron-dense region 0·01 to 0·04 μm thick, and two inner regions both composed of fibrillar material (Pl. 1, fig. B). The outermost fibrillar region is 0·1 to 0·5 μm thick and is composed of closely packed fibrils which are randomly orientated, whilst the inner region is 1·0 to 3·5 μm thick and composed of fine fibrils which usually lie parallel to the shell surface, but are occasionally arranged to produce a 'herring-bone' pattern such as is found in *A. laticollaris*. The electron-dense points in the fibrillar material

have a similar diameter to the fibrils seen in longitudinal cross-section and are probably cross-sections of them.

The pronounced corrugated appearance of the outer region of the shell (Pl. 1, fig. C), is not seen in any other species of *Allogromia* which we have examined.

Allogromia sp. B.

The fine structure of the shell is similar to that found in *A. laticollaris* with the exception of some slight corrugations of the external electron-dense layer. In young schizonts of these animals the fibrillar material and the electron-dense material appear to be distributed at random (Pl. 1, fig. D). As the young schizonts mature, however, the electron-dense particles are arranged in a thin, clearly defined outer layer, that appears to delimit the shell of the animal. The fibrils of the fibrillar layer are then arranged parallel to the surface. The shell of the parent surrounding the schizonts appears to become less compact with the components of both shell layers being dispersed. At this time degeneration of the parent shell is apparent.

Allogromia sp. C.

The fine structure of the shell is similar to that of *A. laticollaris*.

Iridia diaphana

This species is unusual in having two organic shells, one adjacent to the plasma membrane, and the other a tent-like covering at some distance from the animal (Le Calvez, 1936). This latter covering is only associated with sessile forms and is left behind when the animal moves away. It consists mainly of diatoms and other siliceous elements held together by a network of fibrils which are of a similar nature to those found in the main shell.

The true shell of *I. diaphana*, comparable with that of *Allogromia*, is composed of only one fibrillar layer (Pl. 2, fig. A). It varies between 0·8 and 3·3 μm in thickness, with the fibrils usually lying parallel to the shell surface, but occasionally appearing to be swept together to form the 'herring-bone' type of pattern (Pl. 2, fig. B). The electron-dense points seen amongst the fibrils, are probably cross-sections of fibrils, as they have a similar diameter.

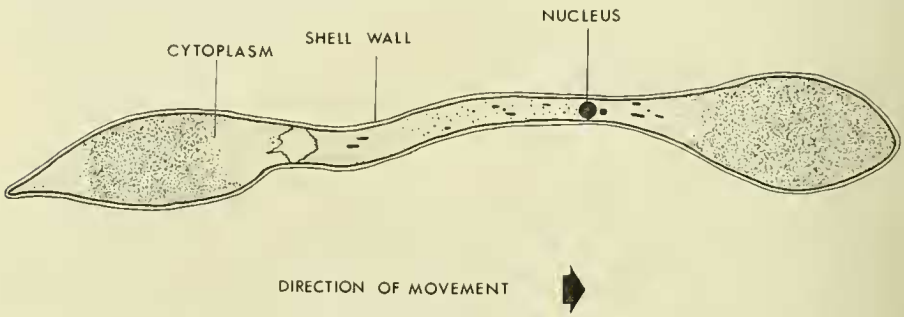

FIG. 1. Diagram of the elongated specimen of *Iridia diaphana*, referred to in the text at the time of fixation.

A specimen of this species was fixed in gluteraldehyde whilst it was moving and changing shape (Text-fig. 1). The structure of the shell in this animal consists of one fibrillar layer (Pl. 2, fig. C) which differs from those in other animals previously discussed here in that constituent fibrils are smaller. These fibrils are arranged at random in both cross and tangential sections.

Boderia turneri

The shell varies between 0·4 and 1·0 μm in thickness. It is composed of only one layer, a fibrillar layer composed of small fibril elements (Pl. 2, fig. D), similar to those seen in the moving specimen of *Iridia diaphana.*

DISCUSSION

The observations reported here throw light on two aspects of shell structure in tectinous foraminifera. Firstly, the evidence suggests that differences exist between the ultrastructural organisation within the shell of actively moving as compared with inactive individuals and secondly, clear differences exist in the ultrastructure of shells of animals which are very similar when examined by optical microscopy alone.

For the purpose of this discussion, the animals examined can be divided into three groups on the basis of their gross shape. Animals belonging to the first group have a relatively constant shape and a fixed aperture position, and include *Allogromia laticollaris*, *Allogromia* sp. A, *Allogromia* sp. B, and *Allogromia* sp. C. In these animals the wall is composed of long thin fibrils arranged parallel to the shell surface and often organized into a herring-bone pattern (Pl. 1, figs A & B). A thin electron-dense layer is usually present at the periphery of the shell. It is worthy of note that adult specimens of *Gromia oviformis*, although not necessarily related to the forms under discussion, also have this type of organic wall (Hedley & Wakefield, 1969).

Animals belonging to the second group usually have a constant shape and aperture position, but under certain conditions they change both. Such a change can take place very rapidly and is typical of *Shepheardella taeniformis* and *Iridia diaphana.* When the shell of these animals is similar to that of the constant shape forms they have a similar ultrastructural organization to them except that the electron-dense peripheral region is either reduced, as in *Shepheardella taeniformis* (Hedley *et al.*, 1967, fig. 3) where it has a minimal thickness, or is absent as in *Iridia diaphana* (Pl. 2, fig. A). When the shell undergoes change in shape as in *Iridia diaphana* the wall has a variable thickness with the fibrils being short, thick, and arranged at random (Pl. 2, fig. C).

Animals belonging to the third group have a constantly changing shape, no apparent aperture and pseudopodia arise from any position of the body surface. A representative of this group is *Boderia turneri* (Pl. 2, fig. D), in which the ultra-structure of the shell resembles that of the moving form of *Iridia diaphana.*

It appears from these observations that differences in the fibrous element of the shells may be correlated with the state of movement in these allogromiids. The short, thick, fibrils in the shell being the form that is associated or correlated with

animals undergoing rapid movement and change in form, whilst the thin, long fibrils are associated with the stable or non-changing state of the shell. In the case of animals capable of changing their shape, for example, *Iridia diaphana*, the short fibrils present in the shells of active animals are presumably transformed into the long fibrils characteristic of inactive forms. These ultrastructural differences between the shells of inactive and active animals of the same species emphasize the need for careful observation of the physical state of the animal at the time of fixation.

The four forms of *Allogromia* studied here appear to be similar in many respects (Table 1). The greatest similarity at the optical level is between *Allogromia laticollaris* and *Allogromia* sp. A. Nevertheless, the fine structure of the shell of *Allogromia* sp. A is seen (Text-fig. 2b, Pl. 1, fig. B) to differ from the other three forms (Text-fig. 2a, Pl. 1, fig. A), by having a compact electron-dense outer region, whilst the remainder of the shell ultrastructure is basically similar (compare Text-figs 2a & b) in all four forms. This feature alone is not sufficient to enable one to differentiate between the forms. However, it would appear to indicate that such details of fine structure of the shell wall (Table 2), could be used as specific characters. A combination of the type of detail listed in Tables 1 & 2 could possibly provide the basis for future identifications.

As the tectinous shell of allogromiid foraminifera lies outside the plasmalemma but remains attached to its outer surface, we regard it as representing a modified glycocalyx as defined by Bennett (1969). He has reviewed the structure and function of various glycocalyces associated with numerous cell types, including protozoa.

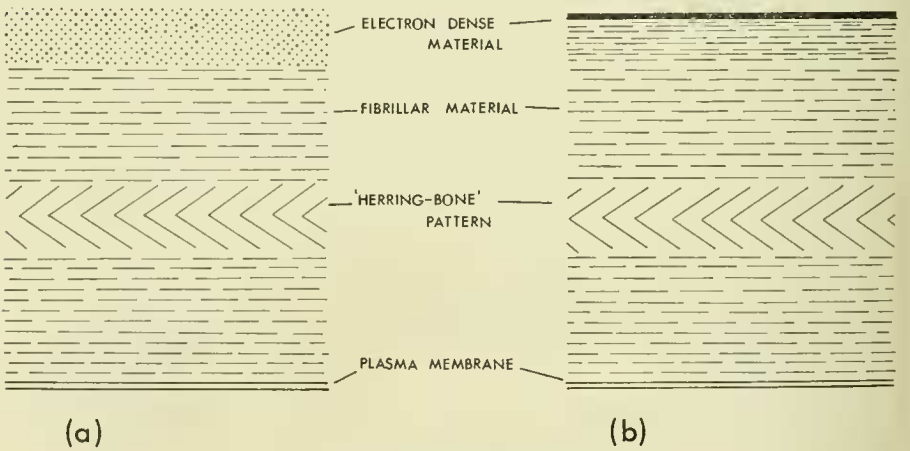

FIG. 2. Diagram of the different arrangements of shell material in forms of *Allogromia*— (a) shell with diffuse electron-dense region; (b) shell with thin compact electron-dense region overlying a region of concentrated fibrillar material. Note that the main fibrillar region is similar in both examples.

From this it would appear that the allogromiid glycocalyx has some affinities with the mucous coat glycocalyces in amoeba.

The permeable nature of such glycocalyces not only allows them to act as effective filters (Bennett, 1963), but in addition they can serve as molecular sieves. For instance, various organic materials can be bound to the strongly anionic polysaccharides of the glycocalyx and they can also be impregnated with inorganic crystals. This property of the glycocalyx, may explain the selective retention of organically bound ferric iron found by Hedley (1960) in the shell of *Gromia oviformis*, and possibly the electron-dense region of the forms described here. Moreover, it also allows the retention of enzymes in the space between the cell and the glycocalyx, which could enable enzymatic changes to occur at the cell surface. This would perhaps facilitate the transformation of the shell ultrastructure in those animals which change rapidly from sessile to active forms.

TABLE 1

Observed differences of four forms of *Allogromia* based on specimens maintained under culture conditions

	Allogromia laticollaris U.S.A.	*Allogromia* sp. A Plymouth England	*Allogromia* sp. B Plymouth England	*Allogromia* sp. C New Zealand
Maximum size (in μm)	400	450	1000	200
General colour	orange-pink	orange-pink	green-yellow	orange-brown
General shape	spherical	pear-like	spherical to elongate *Boderia*-like	spherical
Maturing time*—in weeks	3–4	No information	8	4
Number of schizonts	30–40	No information	50–500	30–50

* Time taken by young schizont to mature

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TABLE 2

Details of the ultrastructure of shells of several tectinous foraminifera

	Allogromia laticollaris	*Allogromia* sp. A	*Allogromia* sp. B		*Allogromia* sp. C	*Myxotheca arenilega*		*Shepheardella taeniformis*	*Iridia diaphana*		*Boderia turneri*	*Gromia oviformis*
Reference	Lengsfeld, 1969 Schwab, 1970 present work	present work	present work		present work	Schwab, 1969 Angell, 1971		Hedley *et al*, 1967	present work		present work	Hedley & Wakefield, 1969
			schizont	adult		schizont	adult		sessile	active		
Electron-dense material—diffuse	+	−	−	+	+	−	+	+	−	−	−	−
Electron-dense material—intense	−	+	−	−	−	N.A.	N.A.	−	−	−	−	+
Long fibril structure	+	+	+	+	+	+	+	+	+	−	−	+
Short fibril structure	−	−	−	−	−	N.A.	N.A.	−	−	+	+	−

N.A. = information not available
\+ = structure present
− = structure absent

PLATE 1

FIG. A. Cross-section of the shell of *Allogromia laticollaris*, showing outer electron-dense layer EDL and inner fibrillar layer F. × 31 300

FIG. B. Section of the shell of *Allogromia* sp. A. Note the intense electron-dense layer, EDL, and the two fibrillar layers F_1 & F_{11}. × 51 200

FIG. C. General view of shell of *Allogromia* sp. A., showing corrugated appearance of outer layers. × 10 000

FIG. D. Cross-section through the shell walls of two adjacent schizonts within the parent shell of *Allogromia* sp. B. × 17 200

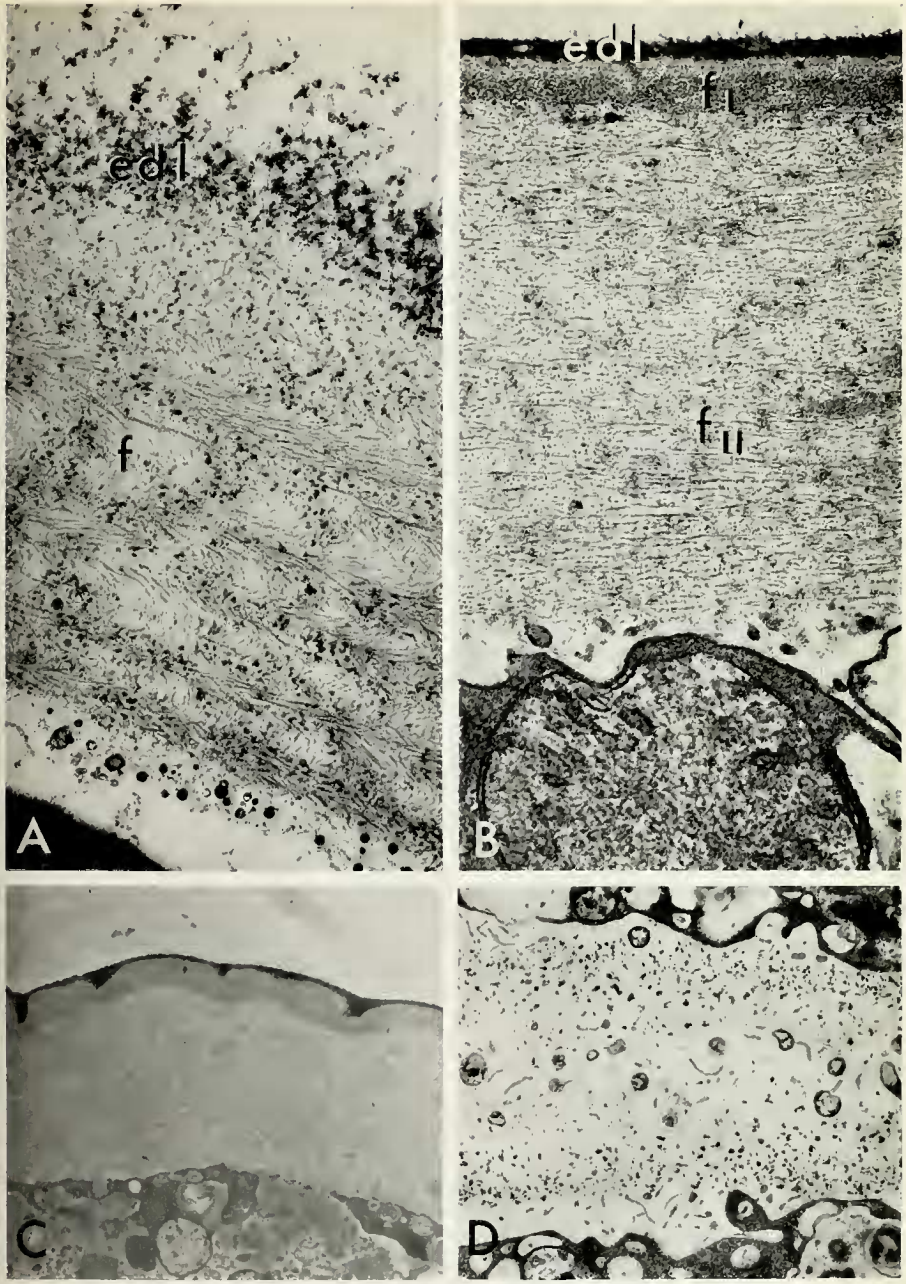

edl
f
A
edl
f_I
f_{II}
B
C
D

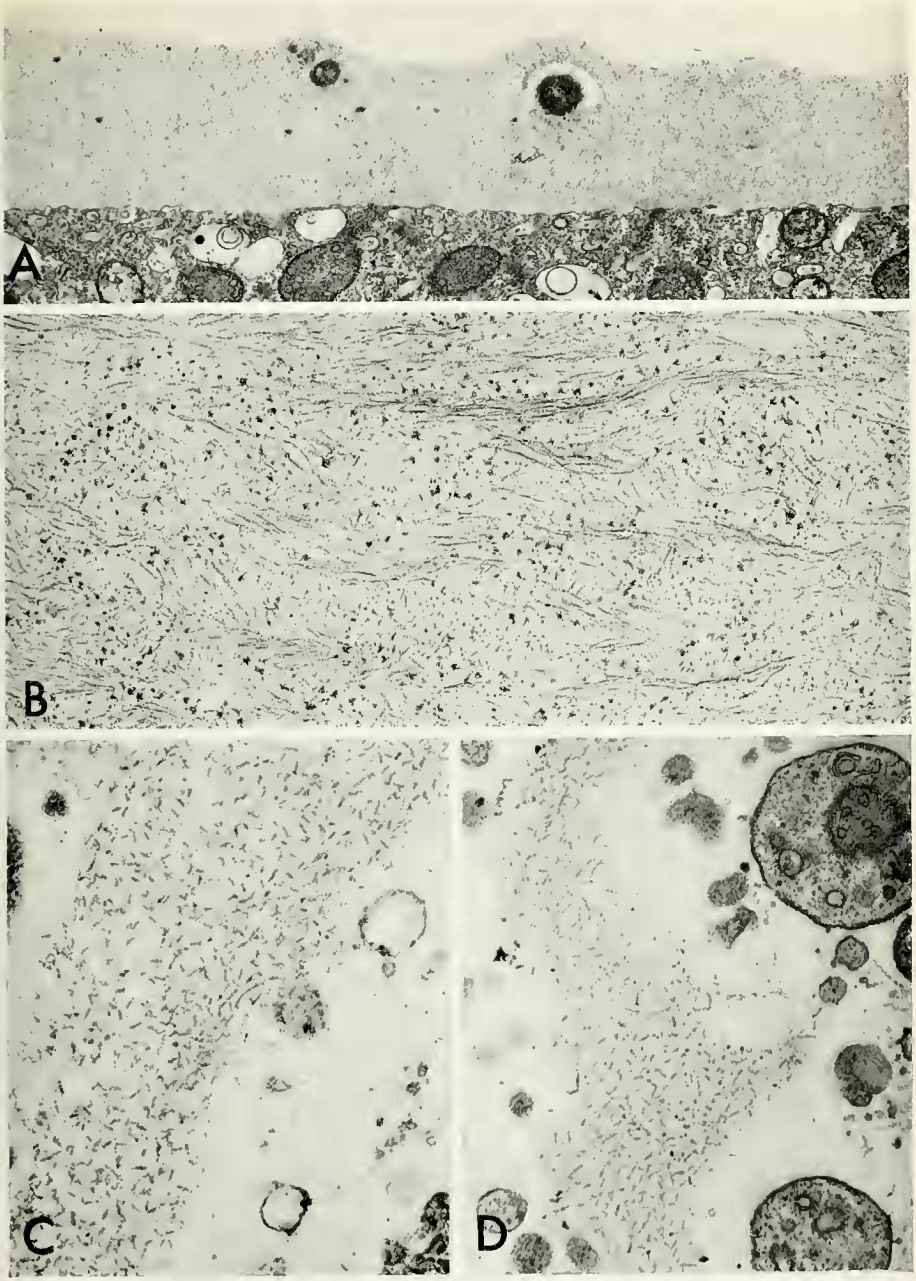
PLATE 2

FIG. A. General view of shell of a sessile *Iridia diaphana* × 12 600

FIG. B. Section of shell of *Iridia diaphana* showing the long fibril organisation associated with inactive animals. × 35 700

FIG. C. A section through the shell of the elongate *Iridia diaphana* Note the shorter fibrils. × 20 700

FIG. D. Cross-section of the shell of *Boderia turneri*. × 21 500


A
B
C
D